

a fluorescent protein operatively linked to at least a second monomer of the fluorescent protein, wherein the propensity of the tandem fluorescent protein to oligomerize is reduced or inhibited as compared to a monomer of the fluorescent protein.

89. (New) The polynucleotide of claim 88, wherein the fluorescent protein is a green fluorescent protein (GFP), a red fluorescent protein (RFP), or a fluorescent protein related to a GFP or an RFP.

90. (New) The polynucleotide of claim 89, wherein the fluorescent protein is a *Discosoma* RFP or a fluorescent protein related to a *Discosoma* RFP.

91. (New) The polynucleotide of claim 90, wherein the *Discosoma* RFP is DsRed, which comprises an amino acid sequence as set forth in SEQ ID NO: 12 or a mutant of SEQ ID NO:12.

92. (New) The polynucleotide of claim 90, wherein the *Discosoma* RFP is a mutant of DsRed, which comprises an amino acid sequence as set forth in SEQ ID NO: 12, further comprising an I125R mutation.

93. (New) The polynucleotide of claim 89, wherein the fluorescent protein is an *Aequorea* GFP, a *Renilla* GFP, a *Phialidium* GFP, or a fluorescent protein related to an *Aequorea* GFP, a *Renilla* GFP, and a *Phialidium* GFP.

94. (New) The polynucleotide of claim 93, wherein the fluorescent protein related to the *Aequorea* GFP is a cyan fluorescent protein (CFP), or a yellow fluorescent protein (YFP), or a spectral variant of the CFP or the YFP.

95. (New) The polynucleotide of claim 93, wherein the fluorescent protein related to the *Aequorea* GFP is an enhanced GFP (EGFP; SEQ ID NO: 4), an enhanced CFP (ECFP; SEQ ID NO: 6), an EYFP-V68L/Q69K (SEQ ID NO: 10), or an enhanced YFP (EYFP; SEQ ID NO: 8).

96. (New) The polynucleotide of claim 88, wherein the fluorescent protein further comprises a mutation of an amino acid residue corresponding to A206, L221, F223, or a combination thereof of SEQ ID NO: 2.

97. (New) The polynucleotide of claim 96, wherein the mutation corresponds to an A206K mutation, an L221K mutation, an F223R mutation, or an L221K and F223R mutation of SEQ ID NO: 2.

98. (New) The polynucleotide of claim 97, wherein the mutation corresponds to an A206K mutation, an L221K mutation, an F223R mutation, or an L221K and F223R mutation of SEQ ID NO: 6 or SEQ ID NO: 10.

99. (New) The polynucleotide of claim 88, wherein the first monomer and the second monomer are operatively linked using a peptide linker.

100. (New) The polynucleotide of claim 99, wherein the fluorescent protein is DsRed, which comprises an amino acid sequence as set forth in SEQ ID NO:12.

101. (New) The polynucleotide of claim 100, wherein the peptide linker has an amino acid sequence as set forth in SEQ ID NO:26.

102. (New) The polynucleotide of claim 88, further comprising at least a third monomer of the fluorescent protein, which is operatively linked to the first monomer or the second monomer.

103. (New) A polynucleotide encoding a fusion protein, wherein the fusion protein comprises the non-oligomerizing tandem fluorescent protein of claim 88 operatively linked to at least one polypeptide of interest.

104. (New) The polynucleotide of claim 103, wherein the non-oligomerizing tandem fluorescent protein is linked to the polypeptide of interest through a peptide bond.

105. (New) The polynucleotide of claim 104, wherein the non-oligomerizing tandem fluorescent protein is linked to the polypeptide of interest through a linker molecule.

106. (New) The polynucleotide of claim 103, wherein the at least one polypeptide of interest comprises a peptide tag.

107. (New) The polynucleotide of claim 106, wherein the peptide tag is a polyhistidine peptide.

108. (New) The polynucleotide of claim 103, wherein the polypeptide of interest is a cellular polypeptide.

109. (New) The polynucleotide of claim 103, wherein the polypeptide of interest is an enzyme, a G-protein, a growth factor receptor, or a transcription factor.

110. (New) The polynucleotide of claim 103, wherein the polypeptide of interest is one of two or more proteins that associate to form a complex.

111. (New) A polynucleotide encoding a tandem non-oligomerizing fluorescent protein, wherein the tandem non-oligomerizing fluorescent protein comprises:

- a) a donor, comprising a first fluorescent protein,
- b) an acceptor, comprising a second fluorescent protein, and
- c) a peptide linker moiety operatively linking the donor and the acceptor,

wherein the first fluorescent protein and second fluorescent protein are different, wherein at least the first fluorescent protein or the second fluorescent protein is a non-oligomerizing tandem fluorescent protein of claim 88, wherein cyclized amino acids of the donor emit light characteristic of the donor, and wherein the donor and the acceptor exhibit fluorescence resonance energy transfer when the donor is excited, and the linker moiety does not substantially emit light to excite the acceptor.

112. (New) The polynucleotide of claim 111, wherein each of the first fluorescent protein and the second fluorescent protein is a non-oligomerizing tandem fluorescent protein.

113. (New) The polynucleotide of claim 111, wherein the non-oligomerizing tandem fluorescent protein comprises a *Discosoma* RFP or a fluorescent protein related to a *Discosoma* RFP.

114. (New) The polynucleotide of claim 113, wherein the *Discosoma* RFP is DsRed, which comprises an amino acid sequence as set forth in SEQ ID NO: 12 or a mutant of SEQ ID NO:12.

115. (New) The polynucleotide of claim 113, wherein the *Discosoma* RFP is a mutant of DsRed, which comprises an amino acid sequence as set forth in SEQ ID NO: 12, further comprising an I125R mutation.

116. (New) The polynucleotide of claim 111, wherein the first fluorescent protein is a non-oligomerizing tandem fluorescent protein, and the second fluorescent protein is a non-oligomerizing fluorescent protein.

117. (New) The polynucleotide of claim 116, wherein the non-oligomerizing fluorescent protein comprises a mutation of an amino acid residue corresponding to A206, L221, F223, or a combination thereof of SEQ ID NO:2.

118. (New) The polynucleotide of claim 117, wherein the mutation corresponds to S65G/S72A/T203Y/H231L in SEQ ID NO:2.

119. (New) The polynucleotide of claim 117, wherein the mutation corresponds to S65G/V68L/Q69K/S72A/T203Y/H231L in SEQ ID NO:2.

120. (New) The polynucleotide of claim 117, wherein the mutation corresponds to K26R/F64L/S65T/Y66W/N146I/M153T/V163A/N164H/H231L in SEQ ID NO: 2.

121. (New) The polynucleotide of claim 117, wherein the mutation corresponds to H148G in SEQ ID NO: 2.

122. (New) A recombinant nucleic acid molecule comprising the polynucleotide of claim 88 operatively linked to at least a second polynucleotide.

123. (New) A recombinant nucleic acid molecule comprising the polynucleotide of claim 111 operatively linked to at least a second polynucleotide.

124. (New) The recombinant nucleic acid molecule of claim 122, wherein the at least second polynucleotide comprises a transcription or translation regulatory element.

125. (New) The recombinant nucleic acid molecule of claim 123, wherein the at least second polynucleotide comprises a transcription or translation regulatory element.

126. (New) The recombinant nucleic acid molecule of claim 122, wherein the at least second polynucleotide encodes a polypeptide of interest.

127. (New) The recombinant nucleic acid molecule of claim 123, wherein the at least second polynucleotide encodes a polypeptide of interest.

128. (New) A vector comprising the polynucleotide of claim 88.

129. (New) A vector comprising the polynucleotide of claim 111.

130. (New) A vector comprising the recombinant nucleic acid molecule of claim 122.

131. (New) A vector comprising the recombinant nucleic acid molecule of claim 123.

132. (New) The vector of claim 130, wherein the vector is an expression vector.

133. (New) The vector of claim 131, wherein the vector is an expression vector.

134. (New) A host cell comprising the polynucleotide of claim 88.

135. (New) A host cell comprising the polynucleotide of claim 111.

136. (New) A host cell comprising the recombinant nucleic acid molecule of claim 122.

137. (New) A host cell comprising the recombinant nucleic acid molecule of claim 123.
138. (New) A kit comprising at least one polynucleotide of claim 88.
139. (New) A kit comprising at least one polynucleotide of claim 111.
140. (New) The kit of claim 138 comprising a plurality of different polynucleotides.
141. (New) The kit of claim 139 comprising a plurality of different polynucleotides.
142. (New) The kit of claim 138 wherein the non-oligomerizing tandem fluorescent protein comprises a fusion protein.
143. (New) The kit of claim 139 wherein the tandem non-oligomerizing fluorescent protein comprises a fusion protein.
144. (New) A kit comprising at least one recombinant nucleic acid molecule of claim 122.
145. (New) A kit comprising at least one recombinant nucleic acid molecule of claim 123.
146. (New) The kit of claim 144 comprising a plurality of different recombinant nucleic acid molecules.
147. (New) The kit of claim 145 comprising a plurality of different recombinant nucleic acid molecules.
148. (New) The kit of claim 144 wherein the at least second polynucleotide comprises a restriction endonuclease recognition site or a recombinase recognition site.
149. (New) The kit of claim 145 wherein the at least second polynucleotide comprises a restriction endonuclease recognition site or a recombinase recognition site.

150. (New) The kit of claim 144 wherein the at least second polynucleotide encodes a polypeptide of interest.

151. (New) The kit of claim 145 wherein the at least second polynucleotide encodes a polypeptide of interest.

152. (New) The kit of claim 144 wherein the at least second polynucleotide encodes a peptide tag.

153. (New) The kit of claim 145 wherein the at least second polynucleotide encodes a peptide tag.

### **THE RESTRICTION REQUIREMENT**

In the Office Action dated September 30, 2002, the Examiner subjected the claims to a restriction requirement. The Examiner grouped the claims into nine groups of allegedly distinct inventions, as outlined below:

- Group I Claims 1-26 and 43-53, which recite a non-oligomerizing tandem fluorescent protein. If this group is elected, the Examiner makes a further requirement for restriction of the claims by requiring that a single fluorescent species (as listed in claims 4, 5, 8 and 9) be elected for prosecution. The Examiner also requires the election of a single polypeptide from claim 22 for prosecution. This is not an election of species requirement.
- Group II Claims 27-42, which recite a polynucleotide encoding a non-oligomerizing tandem fluorescent protein, and related vectors, host cells and kits.
- Group III Claims 54-65, which recite a method of determining the pH of a sample.
- Group IV Claim 66, which recites a method of identifying a sample containing an enzyme.
- Group V Claim 67, which recites a method of determining activity of an enzyme.
- Group VI Claims 68-75, which recite a method of identifying the presence of a molecule in a sample.
- Group VII Claims 68 and 76, which recite a method of identifying the presence of a molecule in a sample.
- Group VIII Claims 77-81, which recite a method of identifying an agent or condition that regulates the activity of an expression control sequence.
- Group IX Claims 82-87 which recite a method of identifying a specific interaction of a first molecule and a second molecule.